Making Sense of Puzzling Genetic Association Studies: A Team Approach

Osteoporosis affects approximately 5 to 8 million Americans older than 50 years of age, and the lifetime risk for osteoporotic fracture is approximately 40% in white women and at least 13% in men (1, 2). Low bone mineral density (BMD) is the major clinical indicator of osteoporotic disease. However, the proportion of fractures attributable to osteoporosis by low BMD is modest, ranging from less than 10% to 44% for specific fracture types and only approximately 15% for all fractures (3). No single factor, such as BMD, can explain osteoporosis, which is a complex metabolic disease caused by actions and interactions among multiple genes, gene products, and environmental factors. These actions and interactions translate to loss of BMD, bone microarchitecture, and bone strength, as well as to nontraumatic fractures. Older women with a parental history of hip fracture have a 2-fold higher risk for hip fracture than those without such a history (4). Family and twin studies indicate that inherited characteristics are responsible for 50% to 80% of the phenotypic variation in traits related to low BMD and fracture (5).

The vitamin D receptor (*VDR*) gene has been a particular target of investigation. Vitamin D regulates bone formation and resorption, intestinal calcium absorption, calcium and phosphate homeostasis, and parathyroid hormone secretion. Vitamin D modulates expression of many genes by first interacting with *VDR*, which then forms complexes that bind to regulator gene regions. Allelic variations in *VDR* might affect the ability to bind vitamin D, which would disrupt vitamin D actions and consequently increase the risk for osteoporosis and fracture. More than 10 years ago, Morrison and colleagues (6) reported the association between *VDR* variants and BMD. This observation initiated a wave of association studies relating *VDR* variants to osteoporosis (7). The biological pathways affected by the common variants, *Fok*I, *Bsm*I, *Apa*I, and *Taq*I, and their related haplotypes remain unclear; however, some studies have suggested that the haplotypes affect pathways leading to variations in bone mass and fracture risk (8). The more recently characterized Cdx2 polymorphism in the *VDR* promoter influences intestinal transcription of the *VDR* gene and may be associated with fracture risk (9, 10), but studies have not consistently shown an association with BMD (11, 12).

It is easy to forget that these studies of associations between genes and diseases use the traditional epidemiologic tools of population studies. Investigators have the same concerns associated with any epidemiologic study: having appropriate design and analytic approaches, sufficient sample size and statistical power, and minimal bias and confounding. Despite hundreds of association studies and retrospective meta-analyses of polymorphisms in more than 30 genes that are associated with BMD and fractures, no convincing conclusions have emerged (13). The *VDR* gene is no exception. To try to address this issue, investigators have reported retrospective meta-analyses of published studies. For example, a recent meta-analysis by Fang and colleagues (14) has shown no relationship between the *VDR Bsm*I or *Taq*I polymorphisms and fracture risk. However, these retrospective meta-analyses typically have significant between-study heterogeneity and biases. Between-study heterogeneity refers to dissimilarity, more than expected by chance, among the estimates of strength of association in the individual studies. Possible causes of dissimilarity include variation in allele frequencies, disease expression, effects of other genetic markers, or disease susceptibility across study samples. Genuine heterogeneity may be difficult to distinguish from the effects of publication or misclassification bias in meta-analyses (15). Lack of standardized genotyping methods and phenotype definitions across studies and publication bias, whereby positive associations are more likely to be published, are major contributing problems to heterogeneity, which in turn makes it difficult to draw conclusions from a body of research.

In this issue, the multicenter association study by Uitterlinden and colleagues (16) has combined individual-patient data from several European prospective cohort and cross-sectional studies. By forming a collaborative consortium, the Genetic Markers for Osteoporosis (GENOMOS) study was able to assess the association of controversial *VDR* polymorphisms and BMD and fracture risks among 23 926 unrelated men and women. These investigators found that the functional Cdx2 polymorphism was associated with a reduced risk for incident fracture, particularly vertebral fracture, but the small effect was of borderline statistical significance. In contrast to findings of some smaller individual association studies, *Fok*I and the *Bsm*I–*Apa*I–*Taq*I haplotype were not associated with BMD or fracture phenotypes.

Genetic Markers for Osteoporosis, the largest collaborative network of studies in osteoporosis genetics, represents a new team approach to quantifying the association between suspected genes and osteoporosis-related outcomes (16). This large-scale prospective approach has key advantages over individual association studies and retrospective meta-analyses. Uitterlinden and colleagues could better minimize between-study heterogeneity and bias by standardizing genotyping methods, outcome definitions, and covariate data collection across studies; controlling for confounding; and improving statistical power to detect modest genetic associations. Moreover, such a large study has greater precision to interpret small effect sizes. When published and unpublished data were included in their col-

laborative study, concern about publication bias was reduced. In genetic association studies, Mendelian randomization also helps to minimize reporting or selection bias. Mendelian randomization refers to the random assortment of alleles from parents to offspring during conception and gamete formation. This leads to population distributions of genetic variants that are generally independent of environmental factors, which often confound nongenetic epidemiologic association studies.

Reports from GENOMOS indicate that multiple genes have a small or modest effect on osteoporotic fracture risk and that several polymorphisms might affect fracture risk through a mechanism at least in part independent of BMD. To date, GENOMOS has found null to modest associations between fracture-related factors and previously controversial polymorphisms in *VDR*, estrogen receptor-α (*ESR1*), and collagen type I-α 1 (*COLIA1*) (16–18). As Uitterlinden and colleagues noted, the Cdx2 polymorphism and other functional polymorphisms in the *VDR* promoter region are in linkage disequilibrium, which means that the association of alleles among the polymorphic sites is not random. This finding implies that Cdx2 or the polymorphisms in linkage disequilibrium with Cdx2 affect fracture risk through a mechanism largely independent of BMD. Likewise, some polymorphisms in *ESR1* and *COLIA1* may be associated with fracture risk, independent of BMD, whereas others are associated with neither BMD nor fracture risk (17, 18).

Large-scale prospective collaborative studies, such as GENOMOS, can identify multiple genes of modest effect and genetic and environmental interactions and provide insights into osteoporosis pathogenesis. Osteoporosis genes are potential future targets for designing new drugs to prevent or treat disease. Susceptibility polymorphisms may aid in assessing persons at high risk or in distinguishing patients who respond to treatment from those who do not. Polymorphisms that are independent of BMD could be used along with BMD to help target preventive therapies to persons at higher risk. The Human Genome Epidemiology Network (www.cdc.gov/genomics/hugenet) is a global collaborative network of consortia formed to prospectively develop and combine knowledge bases on human genetic variants for multiple diseases (19). Recent advances in high-throughput genotyping methods and the influx of validated single-nucleotide polymorphisms in the human genome have now made genome-wide association studies possible (20). In this context, such networks as GENOMOS will facilitate execution and correct interpretation of such studies for osteoporosis and other disease outcomes.

In the next quarter-century, the number of persons affected by osteoporosis and related fractures will double. Understanding the pathogenesis of osteoporosis will require characterizing the interplay among multiple gene variants, gene products, environmental mediators, and bone, which will be an essential step toward discovery of drugs that target the biological mechanism causing osteoporosis. In GENOMOS, Uitterlinden and colleagues show that multicenter collaborative studies are crucial to efficiently and correctly identifying genes involved in osteoporosis and other complex diseases.

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